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A new species of *Volvariella* from India

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Abstract—*Volvariella sathei* sp. nov., collected from Agharkar Research Institute Campus, Pune, is described, illustrated, and compared with morphologically closely related taxa of *Volvariella* and *Volvopluteus*. Diagnostic characters of the new species are the large white fruitbodies, relatively small basidiospores, and cylindric-clavate pleuro- and cheilocystidia. Neighbour-Joining analysis based on ITS sequences confirmed that it is distinct from its closest relative, *V. nivea* and other morphologically similar *Volvariella* species. A key to the Indian species of *Volvariella* and *Volvopluteus* is provided.

Key words—*Agaricales, Basidiomycetes*, macrofungi, taxonomy

Introduction

Volvariella Speg. (*Agaricales*) is well represented in India, and considerable work has been carried out by Pathak et al. (1978) and Pradeep et al. (1998), who have studied the genus and described and/or reported several species for the country. Agaric checklists compiled by Manjula (1983), Natarajan et al. (2005), and Kumaresan (unpublished list) cite 22 species of *Volvariella* including *Volvopluteus* Vizzini et al. as reported from India. A white agaric from Pune is here described as a new *Volvariella* species based on its morphological and molecular characters. Its phylogenetic position has been determined by ITS1+5.8S rDNA+ITS2 sequence analyses.

Materials & methods

Thin handmade sections were made from dried specimens, revived in 10% KOH, and stained in 2% Phloxine. The microscopical characters were studied using an Olympus U-CMAD3 microscope. Approximately 30 basidiospores from sections were measured; the range (extreme values in parentheses) precedes mean spore measurements (in parentheses). Camera lucida diagrams were made using Nikon E200 attached with Nikon Y-IDT prism. Colour terminology follows Kornerup & Wanscher (1978). The type specimen is deposited at Ajrekar Mycological Herbarium (AMH), MACS' Agharkar Research Institute, Pune, India.

Table 1. Sequences used in the phylogenetic analyses.

Species	Voucher No.	GenBank No.
Volvariella bombycina	AJ244	HM562212.1
V. caesiotincta	MA54717	HM562211.1
V. dunensis	SCM3513	JF415140.1
V. hypopithys	TO AV137	HM246492.1
V. lepiotospora	AJ155	HM562214.1
V. nivea	GDGM25489	FJ749127.1
V. nullicystidiata	SP393639	EU920671.1
V. pusilla	AJ51(LOU)	JF415137.1
V. pusilla	TO AV139	HM246494.1
V. sathei	AMH 9436	JN792550
V. strangulata	TO AV141	HM246493.1
V. surrecta	AJ55	HM562213.1
V. taylorii	AJ54	HM562210.1
V. terrea	LUG11010	JF415141.1
V. volvacea	TO AV143	HM246500.1
V. volvacea	ATCC MYA-4696*	HQ999973.1
Volvariella sp.	LOU18924	JF415139.1
Volvopluteus earlei (= V. acystidiata)	TO HG1973	HM246499.1
Vp. earlei (= V. cookei)	TO AV133	HM246496.1
Vp. earlei (≡ V. earlei)	TO AV134	HM246497.1
Vp. earlei (= V. media)	TO HG2001	HM246498.1
Vp. gloiocephalus (≡ V. gloiocephala)	TO AV136	HM246495.1
Schizophyllum commune	IFM 56967*	AB566277.1

*Strain no.

DNA isolation methodology followed Aljanabi & Martinez (1997) with slight modifications. ~300 mg of the holotype gills was washed thoroughly with 99% ethanol and water. DNA was extracted using high salt extraction buffer (NaCl, Tris. HCl and EDTA) and addition of one volume of phenol:chloroform:isoamyl alcohol (25:24:1) and finally precipitated with isopropanol, washed with 70% ethanol, and suspended in Tris-EDTA buffer. The protocol outlined by White et al. (1990) was followed for polymerase chain reaction in Eppendorf Master cycler (Eppendorf, Germany) with universal primers ITS5 and ITS4. Sequencing was performed using BigDye Terminator Cycle Sequencing Kit (Applied Biosystem, UK) as per manufacturer's instructions. The cleaned PCR product was run on ABI 3100 automated DNA sequencer. The raw sequences obtained from ABI sequencer were manually corrected for inconsistencies with the help of Chromas lite software. The DNA sequences obtained in Fasta format were subjected to BLASTn (http://www.ncbi.nlm.nih.gov) analysis.

For phylogenetic analysis, additional related sequences obtained from NCBI DNA sequence database (Table 1) were aligned by CLUSTAL W to generate a phylogenetic tree using MEGA v.5.0 software (Neighbour-joining using Kimura-2 parameter model). The outgroup comprised *Schizophyllum commune* and *Volvopluteus* spp. (recently transferred from *Volvariella*).

Plate 1. *Volvariella sathei*: Basidiomata under natural conditions in Agharkar Research Institute campus. a. Surface view of basidiome. b. Gill view of basidiome. Photo Senthilarasu G.

Taxonomy

Volvariella sathei Senthil., Rahul Sharma & S.K. Singh, **sp. nov.** Plates 1–2

Mycobank MB 561949

Differs from *Volvariella nivea* in smaller subglobose to ovoid basidiospores and smaller pleuro- and cheilocystidia.

Type: India, Maharashtra State, Pune, Agharkar Research Institute Campus (18°52′N 73°83′E), on ground (soil), grass field, 03.08.2009, coll. G. Senthilarasu (**Holotype** AMH 9436; GenBank JN792550).

Etymology: The epithet honors Dr. A.V. Sathe, a leading mycologist in western India.

Pileus 8–9 cm diam., convex; surface white to yellowish white (4A2), fibrillose, shiny with fine radial striations in the margin; Margin thin, eroded; Lamellae free, remote, white becoming sordid, ≤ 10 mm wide, crowded with lamellulae of different lengths, margin concolorous; Stipe 80–105 × 9–13 mm, cylindric, distinctly compressed, tapering towards apex, solid; surface white to cream, glabrous; Pileal context 7 mm thick near stipe, white, unchanging on bruising; Volva free from the stipe, white (4A2), irregularly lobed, thick; Odor fungus like; Taste not recorded.

Basidiospores (5.5)6–6.5 × (4.5)5–5.5, (5.97±0.26 × 5.05±0.26) µm, Q = 1.1, subglobose to ovoid, hyaline with a stramineous thickened wall, smooth; Basidia 20–36 × 5.5–11 µm, cylindric clavate to clavate, tetrasporic; Sterigmata short (≤ 2 µm), slender; Lamella edge heteromorphous; Cheilocystidia scattered, 20–62 × 8–33 µm, cylindric-clavate with broadly rounded apex, thin-walled, easily collapsing; Pleurocystidia scattered, 27–72 × 15–34 µm, cylindric-clavate, mucronate to broadly rounded apex, thin-walled; Lamella trama convergent, consisting of thin walled, hyaline hyphae 5–18 µm diam.; Subhymenium little developed up to 20 µm wide, pseudoparenchymatous; Pileal surface a regular cutis consisting of radially repent to semi erect hyphae, thin-walled, 5–30 µm diam.; Pileal context consisting of tightly interwoven hyphae, 5–30 µm diam., thin-walled; Volva consisting of gelatinous layer, up to 50 µm thick, hyphae thin-walled, up to 33 µm diam., volval elements 20–47 × 15–24 µm, cylindric-clavate; Stipitipellis hyphae made of cylindric, septate hyphae, 5–30 µm diam., terminal elements 30–45 × 12–13 µm, with rounded apex, caulocystidia absent; Clamp-connections absent in all hyphae.

Additional specimens examined: **India**, **Maharashtra state**, Pune, Agharkar Research Institute Campus (18°52′N 73°83′E), on ground (soil), grass field, 21.06.2009, coll. G. Senthilarasu (AMH 9443).

Justo et al. (2010, 2011) reassessed species traditionally assigned to *Pluteaceae* Kotl. & Pouzar based on molecular data. *Volvariella* has been placed outside the pluteoid clade with a new genus, *Volvopluteus,* erected to accommodate species with an ixocutis pileipellis and spores > 11 µm long. Consequently, *Volvariella gloiocephala*, *V. earlei,* and *V. acystidiata* have been transferred to

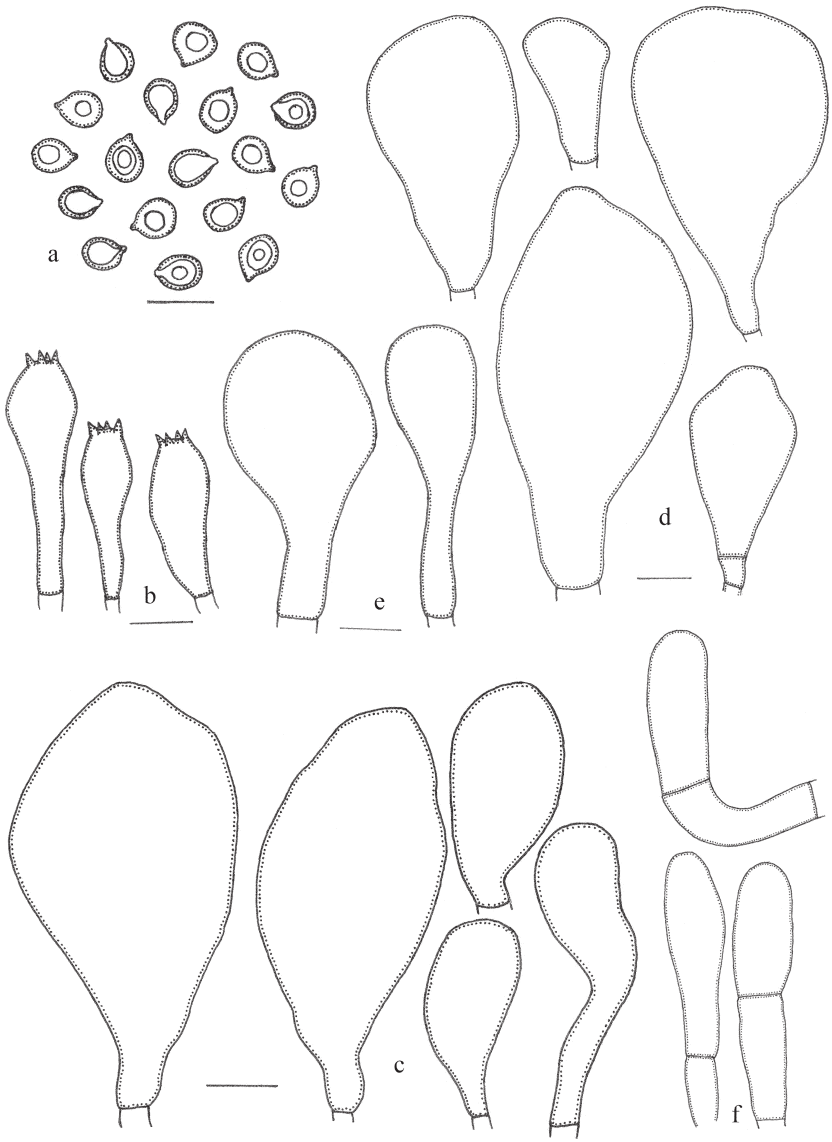

Plate 2. *Volvariella sathei*: a. Basidiospores. b. Basidia. c. Cheilocystidia. d. Pleurocystidia. e. Volval elements. f. Terminal elements of stipitipellis. Scale bar = 10 μm.

Volvopluteus. *Volvariella acystidiata* is considered now a form, as *Volvopluteus earlei* f. *acystidiatus* (N.C. Pathak) Vizzini & Contu (Justo et al. 2011).

Of the 20 species described from India, two belong in *Volvopluteus* (*Vp. gloiocephalus* (DC.) Vizzini et al. and *Vp. earlei* (Murrill) Vizzini et al.), and the remaining species belong in *Volvariella*. *Volvariella sathei* is compared here with morphologically similar taxa belonging to both genera.

Volvopluteus earlei, which also produces white basidiomata, differs in smaller (3–5 cm diam.) size and longer (>11 µm) spores (Justo & Castro 2010; Justo et al. 2010). *Volvopluteus earlei* f. *acystidiatus,* originally described by Pathak (1975) and later by Vizzini & Contu (2010), also closely resembles *V. sathei* but clearly differs by its smaller (20–30 mm diam.) yellowish tinged pileus with pinkish margin, shorter more slender stipe (30–40 × 2–3 mm), larger spores (10.5–16.5 × 7.5–10.5 µm) and basidia (45–70 × 10–13.5), and absence of hymenial cystidia (Justo et al. 2010). Phylogenetically *Volvopluteus* species are clearly distant from *Volvariella* species, including *V. sathei*. There are certain regions within ITS including 5.8S that distinguishes *Volvariella* from *Volvopluteus*.

Volvariella sathei is similar to *Volvariella pusilla* (Pers.) Singer (Pegler 1977, 1983, as *V. parvula*) in the white basidiomata with fibrillose pileus and remote lamellae. However, *V. pusilla* differs in stature with smaller pilei (1.5–3 cm diam.) and stipes (10–40 × 2–4 mm) as well as producing smaller basidia (17–29 × 5–7.5 µm), pleurocystidia (36–53 × 11–17 µm), and cheilocystidia (25–40 × 9–16 µm).

Volvariella nivea T.H. Li & Xiang L. Chen described from China (Li et al. 2009) also produces large, white basidiomata but clearly differs in slightly larger ovoid to broadly ellipsoid spores and considerably larger cheilo- (50–150 × 20–46 µm) and pleurocystidia (60–132 × 19–44 µm).

Volvariella bombycina (Schaeff.) Singer, *V. bombycina* var. *microspora* Dennis, *V. hypopithys* (Fr.) Shaffer, *V. liliputiana* (Henn.) G.C. Rath, and *V. delicatula* (Massee) Manjula are also similar to *V. sathei*. However, *V. bombycina* is lignicolous, and the spores are larger (7.3–9.4 µm in *V. bombycina*; 6–7.5 µm in *V. bombycina* var. *microspora*). *Volvariella hypopithys* has smaller basidiomes (pileus ≤ 5 cm diam.) with squamulose pilei and hairy stipes, while *Volvariella liliputiana* and *V. delicatula* are much smaller (pileus 5–30 mm diam.) than *V. sathei*.

A BLASTn search comparing the AMH 9436 530bp nucleotide sequences (comprising ITS1 = 210bp, 5.8S = 151bp, ITS2 = 157bp) with the Genbank DNA ITS sequence database showed maximum (84%) similarity with *V. terrea* (JF415141.1 query coverage 88%) and 83% similarity with *V. dunensis* (JF415140.1 query coverage 88%). Twenty-two ITS sequences representing 13 *Volvariella* spp., 2 *Volvopluteus* sp., and 1 *Schizophyllum* sp. obtained from Genbank database were aligned using CLUSTAL W with manual adjustments.

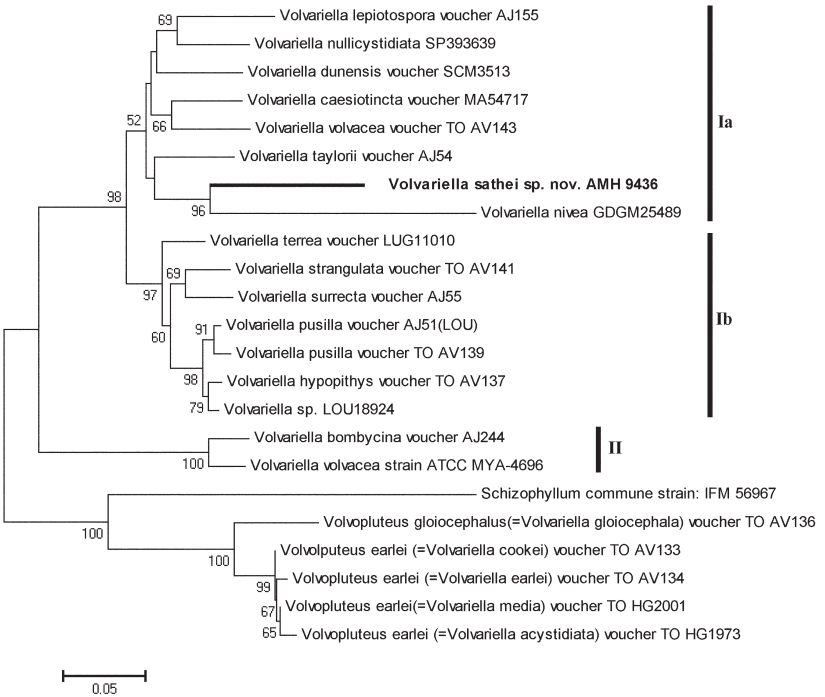
Volvariella lepiotospora voucher AJ155
Volvariella nullicystidiata SP393639
Volvariella dunensis voucher SCM3513
Volvariella caesiotincta voucher MA54717
Volvariella volvacea voucher TO AV143
Volvariella taylorii voucher AJ54
Volvariella sathei sp. nov. AMH 9436
Volvariella nivea GDGM25489
Volvariella terrea voucher LUG11010
Volvariella strangulata voucher TO AV141
Volvariella surrecta voucher AJ55
Volvariella pusilla voucher AJ51(LOU)
Volvariella pusilla voucher TO AV139
Volvariella hypopithys voucher TO AV137
Volvariella sp. LOU18924
Volvariella bombycina voucher AJ244
Volvariella volvacea strain ATCC MYA-4696
Schizophyllum commune strain: IFM 56967
Volvopluteus gloiocephalus(=Volvariella gloiocephala) voucher TO AV136
Volvolputeus earlei (=Volvariella cookei) voucher TO AV133
Volvopluteus earlei (=Volvariella earlei) voucher TO AV134
Volvopluteus earlei(=Volvariella media) voucher TO HG2001
Volvopluteus earlei (=Volvariella acystidiata) voucher TO HG1973
Ia
Ib
II
0.05

Plate 3. Neighbour-joining tree showing phylogenetic position of *Volvariella sathei, Volvariella,* and *Volvopluteus* species. Bootstrap values > 50% are indicated.

The 5.8S region was almost perfectly aligned, but numerous gaps (indels) and misalignments among *Volvariella* species were observed in ITS1 and ITS2 regions. All analysed *Volvariella* species were divided into two main clusters (I and II, Plate 3), with *V. sathei, V. nivea,* and *V. taylorii* together in subclade Ia. Although *V. sathei* and *V. nivea,* both characterized by pure white basidiocarps, are placed together with a strong (96%) bootstrap support, the branch lengths show considerable sequence divergence between them. Our analysis places the two sequences of *Volvariella volvacea* (HM246500.1, TO AV143; HQ999973.1, ATCC MYA-4696) far apart (in completely two different clusters), suggesting ambiguity in deposited sequences.

As Li et al. (2009) has indicated for *V. nivea*, we noted a high degree of variability (including considerable misalignment and several indels) throughout the ITS 1 and ITS 2 regions among *Volvariella* species, but the 5.8S region remained conserved with relatively few changes.

Key to species of *Volvopluteus (Vp.)* and *Volvariella (V.)* in India:

Two newly described species —*Volvariella indica* Pathak et al. (Manjula 1983) and *V. minuta* Kumar et al. (Kumar et al. 2009–10)— and a new record of *V. congolensis* N.C. Pathak (Kumar et al. 2009–10) from India are poorly known and have been excluded from the key.

1. Spores > 11 µm long, pileipellis is an ixocutis 2
1. Spores ≤ 10.5 µm long, pileipellis not an ixocutis. 3

2. Pileus 5–15 cm diam.; spores 11–18 × 6.5–9.5 µm (ivory white to light brownish grey, often darker on the disc) . . . . . *Vp. gloiocephalus*
2. Pileus 3–5 cm diam.; spores 11–15.5 µm long . . . . . *Vp. earlei*

3. Lignicolous, on dead wood, pileus pale yellow then whitish 4
3. Growing on other substrata 7

4. Basidiome robust, > 5 cm diam; volva thick and fleshy 5
4. Basidiome small, < 5 cm diam; volva small, thin 6

5. Spores frequently 10–10.5 µm long . . . . . *V. diplasia*
5. Spores not > 9.5 µm long, volva white . . . . . *V. bombycina*

6. Volva internally rugoso-tomentose; lamellae pale flesh pink; spores 6–7 × 3–4 µm . . . . . *V. glandiformis*
6. Volva smooth; lamellae with brownish tints; spores 6.3–8 × 4–5 µm . . . . *V. thwaitesii*

7. Basidiome growing on other mushrooms . . . . . *V. surrecta*
7. Basidiome growing on soil or other organic substrates 8

8. Basidiome pure white to pinkish white 9
8. Basidiome ash green to brown 13

9. Pileus and stipe distinctly hairy (pileus ≤ 5 cm diam., white; spores 6–7 × 4.5–5 µm) . . . . . *V. hypopithys*
9. Basidiome not hairy, glabrous, subvelutinate to silky fibrillose 10

10. Pileus 8–9 cm diam. white, fibrillose; stipe 80–105 × 9–13 mm . . . . . *V. sathei*
10. Pileus small ≤ 3 cm diam.; stipe short and slender 11

11. Pileus ≤ 1 cm diam, subvelutinate; spores 4.5 × 4 µm . . . . . *V. liliputiana*
11. Pileus ≤ 3 cm diam., glabrous to silky fibrillose 12

12. Spores 5–6 × 3.2–4 µm, pileus at first pinkish white . . . . . *V. delicatula*
12. Spores 4.5–7.5 × 4–6.5 µm, pileus pure white to pale cream (silky smooth to silky fibrillose, volva white, appressed) . . . . . *V. pusilla*

13. Pileus ash-green to grayish brown, volva white . . . . . *V. woodrowiana*
13. Pileus different shades of brown 14

14.Volva, fleshy, rigid, pale to dark brown to blackish brown above, cracking into areolate squamules . . . . . *V. terastia*
14. Volva smooth, not squamose. 15

15. Basidiome large and fleshy; pileus robust ≤ 12 cm diam., tawny brown to grayish brown; stipe 7–20 mm diam.; volva brown 16
15. Basidioma small to medium ≤ 6.5 cm diam.; stipe 2–6 mm diam.; volva grey, white or brown. 18

16. Spores frequently > 9 µm long (6–10.4 × 4.5–7 µm) ..... *V. volvacea*
16. Spores not > 9 µm long. 17
17. Pileus light seal brown at the disc, paling to grayish vinaceous at the margin with fine brown radial striae; spores 4.5–8.6 × 3–5.3 µm ..... *V. cubensis*
17. Pileus bright chestnut brown, spores 5–6.5 × 3.5–4 µm ..... *V. castanea*
18. Pileus grayish; volva grayish 19
18. Pileus brownish; volva brown or white 20
19. Pileus evenly pale to dark grey, slightly darker at the disc; margin not striate ..... *V. taylorii*
19. Pileus evenly grayish with blackish disc; margin or the entire cap deeply grooved ..... *V. nigrodisca*
20. Pileus vinaceous brown at the disc, paler towards the margin; stipe very long, 4–10 cm × 3–6 mm; volva fuliginous brown ..... *V. pseudovolvacea*
20. Pileus ash gray to grayish brown with blackish radial fibrils; stipe short, 4–4.5 cm × 2–4 mm, volva white ..... *V. apalotricha*

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